

CYTOKINES AS TARGETS FOR IMMUNOMODULATION

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ABSTRACT

Immunomodulators, modify the activity of immune function through the impulsive modulation of informational molecules such as cytokines. This may propose an explanation of the effects of herbs on the immune system and other tissues. The immune system is a part of body to find the pathogen by using a specific receptor to create instantly response by the activation of immune components cells, cytokines, chemokines and also release of inflammatory mediator. The aim of this review is to highlight the work on cytokines as targets for immunomodulation and also provides the knowledge on the recent research update. The selection of papers was made using the relevant databases for the biomedical sciences. Cytokines are small glycosylated polypeptides used in the treatment of immune disorders, cancer and various other related diseases. Cytokines are good candidates for plant made pharmaceuticals because the clinical use of cytokines is limited by high production costs. This review provides insights into the future research in this area.

Keywords: Tumor necrosis factor, Interleukins, Immune function.

INTRODUCTIONS

Cytokines, a large group of soluble extracellular proteins or glycoproteins, are key intercellular regulators and mobilizers. Cells of the immune system communicate with one another by releasing and responding to chemical messengers called cytokines. These proteins are secreted by immune cells and act on other cells to coordinate appropriate immune responses. They are now seen to be crucial to innate and adaptive inflammatory responses, cell growth and differentiation, cell death, angiogenesis and developmental as well as repair processes. [1] Cytokine plays a key role in modulation of immune responses. Many different cell types, in addition to immune cells, produce cytokines and express receptors for cytokines. Cell to cell communication is maintained via cytokine networks. [2]

Cytokines include a diverse assortment of interleukins, interferons, and growth factors. One group of cytokines chemically attracts specific cell types. These so-called chemokines are released by cells at a site of injury or infection and call other immune cells to the region to help repair the damage or fight off the invader. Chemokines often play a key role in inflammation and are a promising target for new drugs to help regulate immune responses.

Clinical studies are underway to test its benefits in diseases such as cancer, hepatitis C, and HIV infection and AIDS. Scientists are studying other cytokines to see whether they can also be used to treat diseases. [3, 4]

Functions of cytokines

Cytokines carry out their functions primarily in the immediate cell environment in tissues, although some cytokines may act at a distance by traveling through the bloodstream. Cytokines work by binding to specific receptors on target cell surfaces, stimulating responses in cells that result in the increased or decreased production of proteins. Cytokines are involved as mediator molecules in normal biologic processes. These physiologic functions include growth and differentiation of hematopoietic, lymphoid, and mesenchymal cells, as well as orchestration of host defense mechanisms. However, the unregulated or inappropriate production of particular cytokines may lead to pathological consequences in autoimmune and inflammatory diseases. [5]

Cytokines involved in the activation of cells of the immune system

Cytokines are necessary for the stimulation of T and B lymphocytes. Antigen presenting cells secrete IL-1 which enhances T cell

activation. T_H1 cells produce, amongst other cytokines, IL-2 and IFN- γ which stimulate cytotoxic cells and macrophages leading to cell-mediated immunity. By contrast T_H2 cells produce IL-4 and IL-10 which stimulate B cells and antibody production. IFN- γ inhibits the proliferation of T_H2 cells whereas IL-10 inhibits the development and activity of T_H1 cells.

Cytokines involved in Haemopoiesis

A number of Cytokines stimulate hematopoiesis by acting on hematopoietic progenitor cells. Several members of this family are called colony stimulating factors (G-CSF; GM-CSF). Two other cytokines in this group, IL-3 and IL-7 affect the growth of lymphocyte progenitor cells.

Cytokines that contribute to inflammatory process

Many cytokines contribute to the inflammatory process by activating leucocytes. In addition IL-1, IFN- γ and TNF- α induce the expression of adhesion molecules on endothelial cells causing leucocytes in circulation to adhere to the endothelium. IL-8 acts as a component of chemokines and leads to chemotaxis of leucocytes. IL-1, IL-6 and TNF- α help in acute phase response by acting on a variety of cells.[4-6]

Cytokine as an immunomodulating agent

The term "cytokine" has been used to refer to the immunomodulating agents, such as interleukins and interferons. Biochemists disagree as to which molecules should be termed cytokines and which hormones. As we learn more about each, anatomic and structural distinctions between the two are fading. Classic protein hormones circulate in nanomolar (10^{-9}) concentrations that usually vary by less than one order of magnitude. In contrast, some cytokines (such as IL-6) circulate in picomolar (10^{-12}) concentrations that can increase up to 1,000-fold during trauma or infection. The widespread distribution of cellular sources for cytokines may be a feature that differentiates them from hormones. Virtually all nucleated cells, but especially endo/epithelial cells and resident macrophages are potent producers of IL-1, IL-6, and TNF- α . [7, 8]

Sites of Cytokines Production

In contrast to classical hormones, which are produced at specific sites, the haemopoietic growth factors and other regulatory cytokines are produced by several cell types (Table 1) and in several body sites, either constitutively or after stimulation. This widespread production of cytokines is probably related to the various regulatory roles they are known to play. [9]

Table 1: Cell Types producing Cytokines[9]

| |
|---------------------------|
| T- Cells |
| Bone Marrow Stromal Cells |
| B – Cells |
| Neutrophils |
| Natural Killer Cells |
| Basophils |
| Eosinophils |
| Endothelial Cells |
| Kidney Cells |
| Liver Cells |
| Fibroblasts |
| Reticulum Cells |
| Macrophages |

Cytokines induce their effects in three ways; (1) they act on the cells that produce them (autocrine effect), such as occurs when IL-2 produced by activated T – cells promotes T-cell growth , (2) they effect other cells in their vicinity (paracrine effects), as occurs when IL-7 produced by marrow stromal cells promotes the differentiation of B-cells progenitors in the marrow, and (3) they effect cells systemically (endocrine effect), the best example in this category being IL-1 and TNF which produce the acute phase response during inflammation . [10, 11]

Categories of cytokines [12-15]

Cytokines can be grouped into different categories based on their functions or their source but it is important to remember that because they can be produced by many different cells and act on many different cells, any attempt to categorize them will be subject to limitations.

A. Mediators of natural immunity

Cytokines that play a major role in the innate immune system include: TNF- α , IL-1, IL-10, IL-12, type I interferons (IFN- α and IFN- β), IFN- γ , and chemokines.

1. TNF- α

Tumor necrosis factor alpha is produced by activated macrophages in response to microbes, especially the lipopolysaccharide (LPS) of Gram negative bacteria. It is an important mediator of acute inflammation. It mediates the recruitment of neutrophils and macrophages to sites of infection by stimulating endothelial cells to produce adhesion molecules and by producing chemokines which are chemotactic cytokines. TNF- α also acts on the hypothalamus to produce fever and it promotes the production of acute phase proteins.

2. IL-1

Interleukin 1 is another inflammatory cytokine produced by activated macrophages. Its effects are similar to that of TNF- α and it also helps to activate T cells.

3. IL-10

Interleukin 10 is produced by activated macrophages and Th2 cells. It is predominantly an inhibitory cytokine. It inhibits production of IFN- γ by Th1 cells, which shifts immune responses toward a Th2 type. It also inhibits cytokine production by activated macrophages and the expression of class II MHC and co-stimulatory molecules on macrophages, resulting in a dampening of immune responses.

4. IL-12

Interleukin 12 is produced by activated macrophages and dendritic cells. It stimulates the production of IFN- γ and induces the differentiation of Th cells to become Th1 cells. In addition, it enhances the cytolytic functions of T and NK cells.

5. Type I interferons

Type I interferon (IFN- α and IFN- β) are produced by many cell types and they function to inhibit viral replication in cells. Type I interferons also activate NK cells.

6. INF- γ

Interferon gamma is an important cytokine produced by primarily by Th1 cells, although it can also be produced by T cells and NK cells to a lesser extent. It has numerous functions in both the innate and adaptive immune systems.

7. Chemokines

Chemokines are chemotactic cytokines produced by many kinds of leukocytes and other cell types. They represent a large family of molecules that function to recruit leukocytes to sites of infection and play a role in lymphocyte trafficking.

B. Mediators of adaptive immunity

Cytokines that play a major role in the adaptive immune system include: IL-2, IL-4, IL-5, TGF- β , IL-10 and IFN- γ .

1. IL-2

Interleukin 2 is produced by Th cells, although it can also be produced by Tc cells to a lesser extent. It is the major growth factor for T cells. It also promotes the growth of B cells and can activate NK cells and monocytes as depicted in. IL-2 acts on T cells in an autocrine fashion. Activation of T cells results in expression of IL-2R and the production of IL-2. The IL-2 binds to the IL-R and promotes cell division. When the T cells are no longer being stimulated by antigen, the IL-2R will eventually decay and the proliferative phase ends

2. IL-4

Interleukin 4 is produced by macrophages and Th2 cells. It stimulates the development of Th2 cells from naïve Th cells and it promotes the growth of differentiated Th2 cells resulting in the production of an antibody response. It also stimulates Ig class switching to the IgE isotype.

3. IL-5

Interleukin 5 is produced by Th2 cells and it functions to promote the growth and differentiation of B cells and eosinophiles. It also activates mature eosinophiles.

4. TGF- β

Transforming growth factor beta is produced by T cells and many other cell types. It is primarily an inhibitory cytokine. It inhibits the proliferation of T cells and the activation of macrophages.

C. Stimulators of hematopoiesis

Some cytokines stimulate the differentiation of hematopoietic cells. These include GM-CSF which promotes the differentiation of bone marrow progenitors, M-CSF, which promotes growth and differentiation of progenitors into monocytes and macrophages.

Cytokine receptors

In recent years, the cytokine receptors have come to demand the attention of more investigators than cytokines themselves, partly because of their remarkable characteristics, and partly because a deficiency of cytokine receptors has now been directly linked to certain debilitating immunodeficiency states. In this regard, and also because the redundancy and pleiomorphism of cytokines are, in fact, a consequence of their homologous receptors, many authorities think that a classification of cytokine receptors would be more clinically and experimentally useful.

A classification of cytokine receptors based on their three-dimensional structure has, therefore, been attempted. Such a classification, though seemingly cumbersome, provides several unique perspectives for attractive pharmacotherapeutic targets.[13]

- Immunoglobulin (Ig) super family, which are ubiquitously present throughout several cells and tissues of the vertebrate body, and share structural homology with immunoglobulins (antibodies), cell adhesion molecules, and even some cytokines. Examples: IL-1 receptor types.

- Interferon (type 2) family, whose members are receptors for IFN β and γ .
- Tumor necrosis factors (TNF) (type 3) family, whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligands like CD40, CD27 and CD30, besides the ligands on which the family is named (TNF).
- Seven trans membrane helix family, the ubiquitous receptor type of the animal kingdom. All G protein-coupled receptors (for hormones and neurotransmitters) belong to this family. Chemokine receptors, two of which act as binding proteins for HIV, also belong to this family.

Cytokines are currently being used clinically as biological response modifiers for the treatment of various disorders. The term cytokine is a general term used to describe a large group of proteins but there are other terms that are commonly used to describe particular kinds of cytokines. [16]

- Monokines, cytokines produced by mononuclear phagocytic cells
- Lymphokines, cytokines produced by activated lymphocytes, especially Th cells
- Interleukins, cytokines that act as mediators between leukocytes

Table 2: Features of cytokines[12-15]

| Cytokine | Cell Source | Cell Target | Primary Effects |
|-------------|--|--|---|
| IL-1 | Monocytes Macrophages Fibroblasts Epithelial cells Endothelial cells Astrocytes | T cells; B cells Endothelial cells Hypothalamus Liver | Co stimulatory molecule Activation (inflammation) Fever Acute phase reactants |
| IL-2 | T cells; NK cells | T cells B cells Monocytes | Growth Growth Activation |
| IL-3 | T cells | Bone marrow progenitors | Growth and differentiation |
| IL-4 | T cells | Naive T cells T cells B cells | Differentiation into a T _H 2 cell Growth Activation and growth; Isotype switching to IgE |
| IL-5 | T cells | B cells Eosinophils | Growth and activation |
| IL-6 | T cells; Macrophages; Fibroblasts | T cells; B cells Mature B cells Liver | Co stimulatory molecule Growth (in humans) Acute phase reactants |
| IL-8 family | Macrophages; Epithelial cells; Platelets | Neutrophils | Activation and chemotaxis |
| IL-10 | T cells (T _H 2) | Macrophages T cells | Inhibits APC activity Inhibits cytokine production |
| IL-12 | Macrophages; NK cells | Naive T cells | Differentiation into a T _H 1 cell |
| IFN-gamma | T cells; NK cells | Monocytes Endothelial cells Many tissue cells - especially macrophages | Activation Activation Increased class I and II MHC |
| TGF-beta | T cells; Macrophages | T cells Macrophages | Inhibits activation and growth Inhibits activation |
| GM-CSF | T cells; Macrophages; Endothelial cells, Fibroblasts | Bone marrow progenitors | Growth and differentiation |
| TNF-alpha | Macrophages; T cells | Similar to IL-1 | Similar to IL-1 |

IL = interleukin GM-CSF = granulocyte-macrophage colony stimulating factor

IFN = interferon TNF = tumor necrosis factor

TGF = transforming growth factor

Cytokines are not typically stored as preformed proteins. Rather their synthesis is initiated by gene transcription and their mRNAs are short lived. They are produced as needed in immune responses. Many individual cytokines are produced by many cell types and act on many cell types (i.e., they are pleotropic) and in many cases cytokines have similar actions (i.e., they are redundant). Redundancy is due to the nature of the cytokine receptors. Receptors for cytokines are heterodimers (sometimes heterotrimers) that can be grouped into families in which one subunit is common to all members of a given family. Since the subunit common to all members of the family functions in binding cytokine and in signal transduction, a receptor for one cytokine can often respond to another cytokine in the same family. Thus, an individual lacking IL-2, for example, is not adversely affected because other cytokines (IL-15, IL-7, IL-9, etc.) assume its function. Similarly, a mutation in a cytokine receptor subunit other than the one in common often has little effect. On the other hand, a mutation in the common subunit has profound effects. For example, a mutation in the gene for the IL-2R gamma subunit causes human X-linked severe combined immunodeficiency (XSCID) characterized by a complete or nearly complete T and B cell defects. [17]

One cytokine often influences the synthesis of other cytokines. They can produce cascades, or enhance or suppress production of other cytokines. In addition, they can often influence the action of other cytokines. The effects can be:

- Antagonistic
- Additive
- Synergistic
- Cytokines bind to specific receptors on target cells with high affinity and the cells that respond to a cytokine are either: 1) the same cell that secreted cytokine (autocrine); 2) a nearby cell (paracrine) or 3) a distant cell reached through the circulation (endocrine). Cellular responses to cytokines are generally slow (hours) because they require new mRNA and protein synthesis.[18]

Cytokine and disease

Adverse effects of cytokines have been linked to many disease states and conditions ranging from major depression [18] and Alzheimer's disease [20] to cancer [21] with levels either being elevated or

changed. Over secretion of cytokines can trigger a dangerous syndrome known as a cytokine storm; this may have been the cause of severe adverse events during a clinical trial of TGN1412. Plasma levels of various cytokines may give information on the presence, or even predictive value of inflammatory processes involved in autoimmune diseases such as rheumatoid arthritis,[22] as well as immunomodulatory effects of foods or drugs.[23] In addition, elevated levels of IL-7, an important cytokine involved in T cell homeostasis, have been detected in the plasma of HIV-infected patients.[24]

Cytokine production is increased with obesity, as macrophage numbers in adipose tissue are increased; adipose tissue macrophages contribute to as much as 50% of adipose tissue TNF- α production. Moreover, chronic inflammatory diseases are associated with overproduction of cytokines, including IL-1 β , IL-6, and TNF- α .[25] Antioxidants have been shown to curb the production of IL-1 β , IL-6, and TNF- α in healthy human after exercise. Vitamin E, a potent, lipid-soluble, and chain-breaking antioxidant, inhibits or suppresses cytokine production, particularly in IL-1 β , IL-6, and IL-10.[26]

Pro-inflammatory cytokines such as TNF- α impair insulin action in peripheral tissue and contribute directly to obesity-linked insulin resistance, while IL-6 changes insulin sensitivity, influencing glucose metabolism. [27] Tumor necrosis factor- α is an especially important cytokine in the development of steatohepatitis and other forms of liver injuries as well as apoptosis. Elevation of TNF- α level could harm the body during the effector phase of the immune response.[28, 29] The Kugelmas et al. study determined that alcoholic steatohepatitis (AH) is due at least in part to abnormal cytokine metabolism, with increased serum concentrations of TNF and TNF-induced cytokines present. Moreover, nonalcoholic steatohepatitis (NASH) is also associated with increased serum TNF concentrations. Interleukin-1 β , stimulated by TNF- α and other cytokines, is associated with the pathogenesis of diseases causing bone loss such as osteoporosis, cancer-induced osteolysis, rheumatoid arthritis, and osteolysis of orthopedic implants.[26]

Elevated levels of TNF- α and IL-6 have been associated with arthritic disease such as gouty arthritis, rheumatoid arthritis, and psoriatic arthritis. In patients with rheumatoid arthritis, release of pro-inflammatory cytokines led to bone loss around the affected joints; the concern is that pro-inflammatory cytokines are linked to osteoporosis.[26] Elevation of IL-1 β , IL-6, IL-8, and TNF- α have been found in patients with uncontrolled diabetes. Studies on rats have shown that vitamin E (in the forms of palm-derived tocotrienol and α -tocopherol) may prevent osteoporosis by suppressing IL-1 and IL-6. Vitamin E neutralizes free radicals that generally activate transcription factor NF κ B; this transcription factor stimulates the production of bone resorbing cytokines.

Vitamin E also inhibits COX-2, which is the enzyme involved in inflammatory reactions, post-translation.[26, 30] Vitamin E inhibits the enzymatic activity and biological function of macrophage migration inhibitory factor (MIF), subsequently blocking MIF-induced IL-6 production and reducing neuropathic pain. [31] Like vitamin E, vitamin C inhibits the activation of transcription factor NF κ B by blocking the degradation of the inhibitor of NF κ B in a process mediated by TNF- α .[32, 33]

CONCLUSIONS

Emerging facts about the immune system and immune system related disorders start up new perspectives for the implication of cytokine therapy and opens a potentially large market. Phytotherapy furnishes a potential therapeutic modality for the treatment of many differing conditions involving cytokines via immunomodulation. Altered cytokine biosynthesis characterized by the formation of various oxygen intermediates and this increases cytokine transcription with activation of immune effectors' cells, improve immune function and survival. Many agents that act on above pathways are being tested. The clinical immunologist, while still relying predominantly on pharmacologic means, now has the reagents to afford more selective and specific means of immunosuppression, immunomodulation, and immunotherapy. There are several new directions for study firstly the hormones that

modify the function of immune system need to be better characterized. As this will provide better understanding. We should also explore the possibility of optimizing hormonal concentrations in plasma in an attempt to promote immune system function. Secondly small or more changes or parameters may increase the biological susceptibility to infectious diseases far greater magnitude than a small change in a single parameter of immune function. It will be important for immunologist to define the significance of alterations in the function of the numerous components of immune system. Application of this new science to such common diseases as allergic asthma offers the possibility of changing the natural history of allergic and immunologic diseases.

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